Supplemental Document

Biomedical Optics EXPRESS

In vivo assessment of inflammatory bowel disease in rats with ultrahigh-resolution colonoscopic OCT: supplement

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Supplement DOI: https://doi.org/10.6084/m9.figshare.19317650

Parent Article DOI: https://doi.org/10.1364/BOE.453396

SUPPLEMENTARY MATERIALS

In vivo assessment of inflammatory bowel disease in rats with ultrahigh-resolution colonoscopic OCT

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Rat IBD model and experimental design

TABLE S1. Experimental design

Time (days)	D0	D3	D8	D13
Procedures	OCT TNBS challenge	OCT	OCT	OCT
IBD group	U1-4	U1-4	U1-4	U1-4 (Sacrificed)
	U5	U5	U5 (Sacrificed)	
	U6-7	U6-7 (Sacrificed)		
Control group	C1-3	C1-3	C1-3	C1-3 (Sacrificed)
	C4 (Sacrificed)			

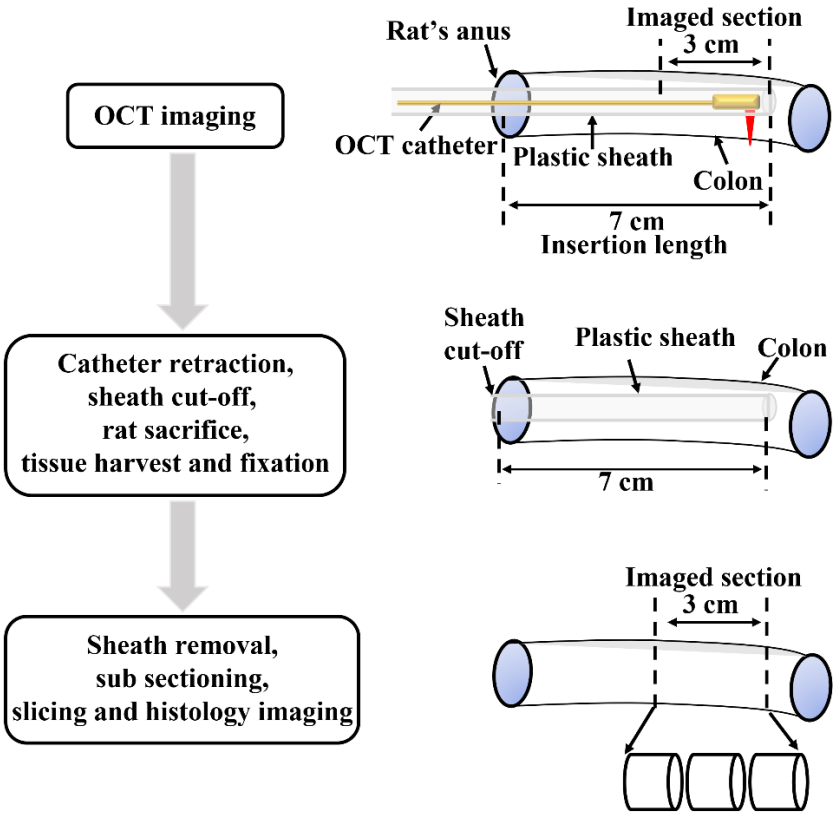

Figure S1. The flowchart and corresponding schematics to illustrate the registration procedures between OCT and histology.

OCT versus histology correlation study

The high resolving power of 800-nm colonoscopic OCT enables the accurate identification of the colonic mucosal layer, thus providing a chance to accurately and reproducibly quantify its thickness in OCT images. To study the correlation between OCT and histology, we totally harvested 11 rat colons. From each rat colon, we acquired 3-4 small colon sections for

histological processing. Totally 39 correlated OCT image and histology micrograph pairs were identified from 41 colon sections. Two investigators (i.e., WY and DC) measured the averaged thickness of mucosa layer on OCT and histological micrographs separately and blindly. For this measurement, we developed a freeform (drawing) based method with MATLAB and manually segmented the mucosa layer on the OCT images and corresponding histology micrographs (n=39). A significant correlation ($r=0.98$, $p<0.001$) was found for the mucosa thicknesses measured from OCT 2D cross-sections versus those from the correlated histology (see Fig. S2). It is noticed that the thicknesses measured from histology micrographs are systematically smaller (by about 13.95% with a standard deviation of 3.39%) than those measured from OCT images due to tissue shrinkage during fixation by formalin.

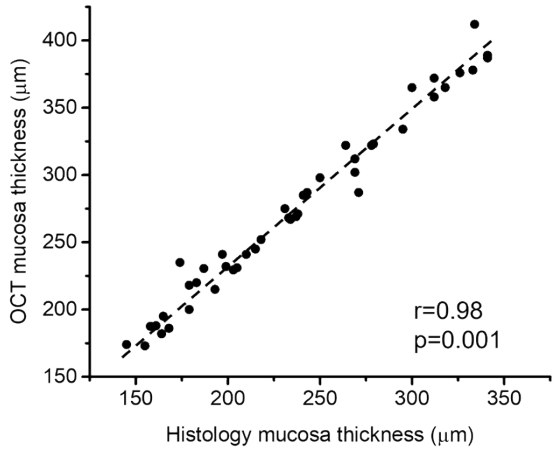

Figure S2. The correlation of the colonic mucosal thickness measured from OCT images and corresponding histological micrographs (data from 39 pairs of images acquired from 11 rats).

Disease activity index (DAI) of rat IBD group and control group

DAI is a common parameter for scoring the disease activity at different severity of IBD (28, 32, 41). A higher DAI score indicates an advanced colitis condition. This reflects in the significantly higher DAI scores of the IBD group than that of the control group, indicating substantial loss of body weight, diarrhea, and bloody stool (see Table S2, Fig. S3).

Disease activity index score sheet for rat IBD model

TABLE S2. Disease activity index (DAI) score sheet for IBD

	0	1	2	3	4
Weight loss	None	<10%	10-15%	15-20%	>20%
Stool consistency	Normal		Loose stool		Diarrhea
Bleeding	None		Focal occult blood		Gross blood

To facilitate the analysis, the degree of disease activity is grouped into four categories based on the DAI scores, such as category 0 with a DAI score 0, category I with DAI score 1-2, category II of DAI score 2-6, and category III of DAI score 6-10. In our study, category III suggests a severe disease and colitis, category II indicates a moderate IBD condition, and category I implies a mild disease, while category 0 means normal.

Figure S3 shows that the current rat model developed a severe IBD after the TNBS administration, and the most severe condition was reached at about day 3. The IBD model then progressed to a moderate condition. From about day 10, the IBD group proceeded into a remission stage with a mild disease condition. These observations are consistent with the previous studies on TNBS-based small animal IBD models (27, 28, 32).

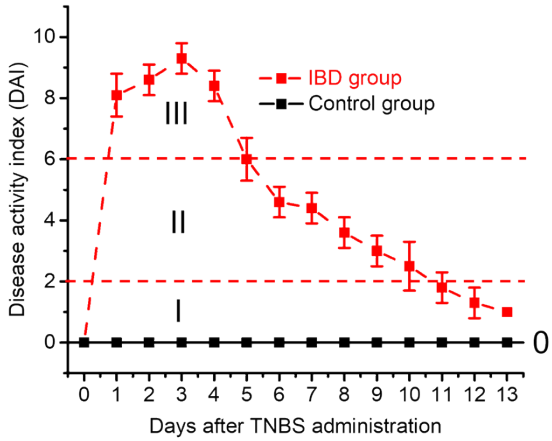

Figure S3. DAI scores of IBD and control groups during the 14-day study period. Severity of IBD was grouped into 4 categories according to the DAI scores (0, I, II, and III).

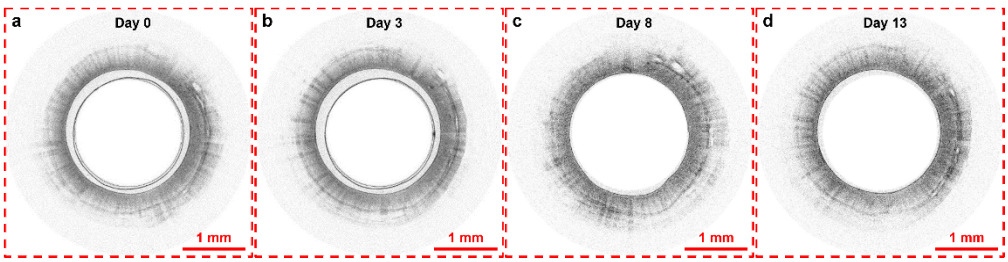

Figure S4. Representative OCT images of the control group at day0, day3, day8 and day13. Representative OCT images of rat C1 acquired from approximately the same location in colon at 4 different time points, i.e., day 0, day 3, day 8, day 13, during the 14-day study period. Comparing with the IBD group (Figs. 3 and 4), no microstructural changes in the colon were observed in control group.

Correlating the degree of colitis activity with the OCT derived quantitative metrics

To study the colitis activity in rat IBD model, two OCT metrics, i.e., the mean thickness and mean attenuation coefficient of the mucosal layer, are quantified from 4 symmetric longitudinal (yz) images generated from the 3D colonic intensity and attenuation volumes of each rat acquired at each time point (see Fig. S5a). Same freeform based method was used to manually segment the mucosa layer on the OCT longitudinal intensity and attenuation images.

Figure S5b shows the colonic mucosa thicknesses (normalized with the baseline measurement at D0 for each rat) and attenuation coefficients in each DAI category for both IBD and control groups. A significant increase of mucosa thickness (by about 80% from category 0 to category

III) and decrease in attenuation values (by approximately 1 mm^{-1} from category 0 to category III) are observed along with the progression of colitis in the IBD group, while relatively constant OCT measurements are found in the control group during the study period. Figures S5c and d show that the colitis activity in rats (as indicated with DAI scores) was strongly associated with the increase in colonic mucosa thickness (r=0.92, p<0.001) and the decrease of mucosal attenuation coefficient (r=0.93, p<0.001), respectively.

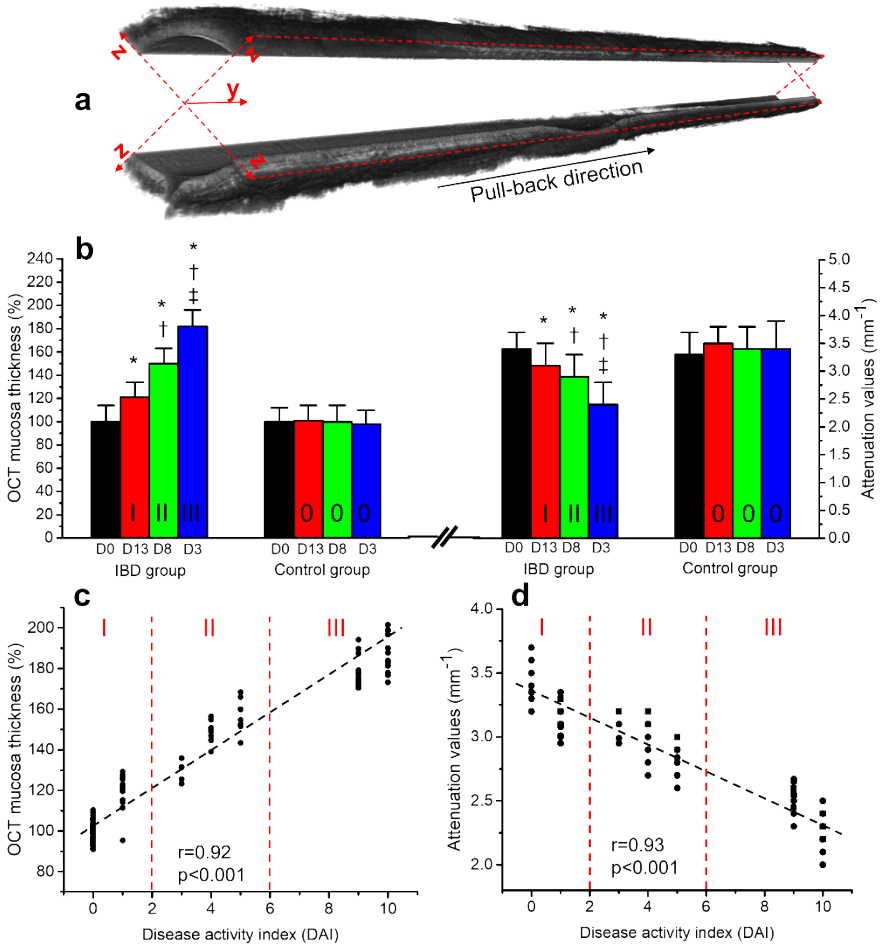

Figure S5. (a) Four longitudinal intensity (yz) images generated from the 3D intensity volume for the quantification of mean mucosa thickness. Similar method was used to quantify the mean attenuation value from the volumetric attenuation dataset. (b) the normalized OCT mucosa thickness and attenuation coefficients in each DAI category for 11 rats. For IBD group, n=7 for D0 (category 0), n=4 for D13 (category I), n=5 for D8 (category II), n=7 for D3 (category III). For control group, n=4 for D0, n=3 for D13, D8, and D3. Asterisks indicate P<0.001 for the comparison with category 0. Daggers indicate P<0.001 for the comparison with category I. Double daggers indicate P<0.001 for the comparison with category II. (c, d) The relationship between the degree of colitis activity of IBD and quantitative OCT metrics, IBD and control groups have been combined (n=36).